Supplemental Information

Mitochondria Bound to Lipid Droplets Have Unique Bioenergetics, Composition, and Dynamics That Support Lipid Droplet Expansion

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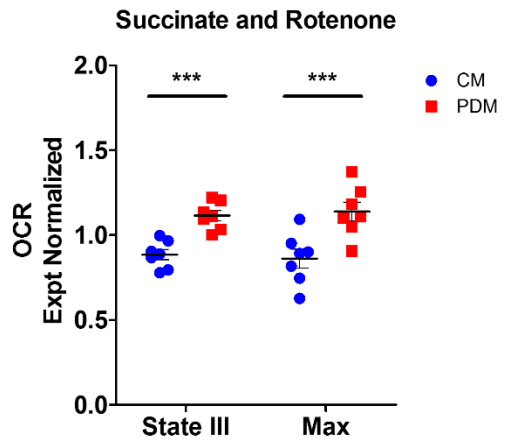

Figure S1, related to Figure 2.

Quantification of respiratory states driven by succinate/rotenone in cytoplasmic (blue) and peridroplet (red) mitochondria. State III quantifies respiration driven by ATP synthesis and maximal respiration quantifies maximal electron transport activity induced by the chemical uncoupler FCCP. 6 technical replicates per group. N = 7 independent isolations. For each individual experiment, average OCR values of CM and PDM were normalized to the average OCR of all mitochondria (see Quantification and Statistical Analysis for complete equations). Data are expressed as means ± SEM. *** $p < 0.0001$.

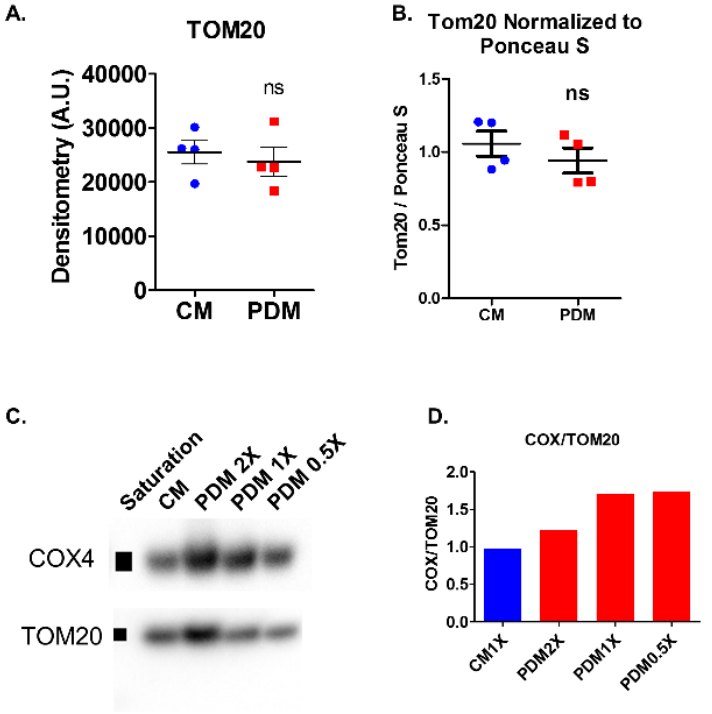
A.
TOM20
Densitometry (A.U.)
40000
30000
20000
10000
0
ns
CM
PDM
B.
Tom20 Normalized to Ponceau S
Tom20 / Ponceau S
1.5
1.0
0.5
0.0
ns
CM
PDM
C.
Saturation
CM
PDM 2X
PDM 1X
PDM 0.5X
COX4
TOM20
D.
COX/TOM20
COX/TOM20
2.0
1.5
1.0
0.5
0.0
CM1X
PDM2X
PDM1X
PDM0.5X
E.
COX4
Saturation
F.
COX4
Saturation
250
200
150
Gray Value
0.0
0.2
0.4
0.6
0.8
Distance (cm)
G.
MW
kDa 250
150
100
75
50
37
25
20
15
10
ATP5A1
COX4

Figure S2, related to Figure 3.

A. TOM20 densitometry from Western blot analysis of isolated mitochondria. N = 4 independent isolations. Experiment normalized data are expressed as means ± SEM. ns $p>0.05$.

B. TOM20 densitometry normalized to Ponceau S staining of SDS-PAGE blots of isolated mitochondria. N = 4 independent isolations. Experiment normalized data are expressed as means ± SEM. ns $p>0.05$.

C. Dilution Western blot of isolated mitochondria. One microgram of CM was compared to 2 microgram PDM (PDM 2X), 1 microgram of PDM (PDM 1X), 0.5 micrograms of PDM (0.5X), and saturating signal intensity.

D. Quantification of COX4/TOM20 ratio at different dilutions. Note that COX/Tom20 ratio is elevated in PDM independent of the amount of protein loaded.

E-F. Linescan histogram of COX4 immunostained band in isolated mitochondria subjected to western blot analysis compared to saturating signal intensity. Note that COX4 band did not exceed saturation (255 A.U. in 8-bit image).

G. Full western blot membrane of COX4 and ATP synthase antibodies used for immunofluorescence experiments. Both COX4 and ATP synthase antibodies produced robust bands at or near the predicted molecular weights (37 kDa and 53 kDA, respectively). There were moderate faint bands above 150 kDa in ATP synthase blot likely representing multimers that were not fully denatured by SDS.

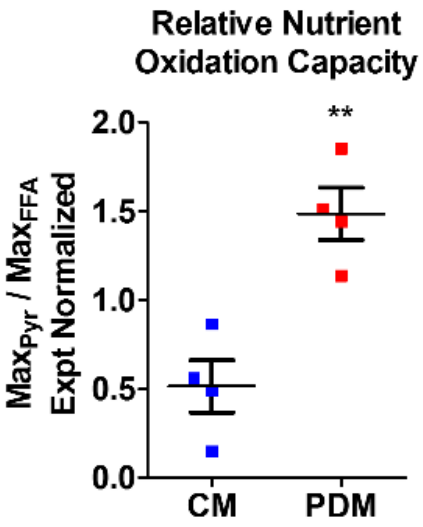

Figure S3, related to Figure 4.

Maximal respiration fueled by pyruvate normalized to maximal respiration fueled by palmitoyl-carnitine (Max_{Pyr}/Max_{PC}) in isolated cytoplasmic (CM) and peridroplet (PDM) mitochondria. Fuels were provided to the exact same mitochondrial preparations assayed on parallels wells in same seahorse plate. N = 4 independent experiments with 4-6 technical replicates per group. Experiment normalized data are expressed as means ± SEM. ** $p < 0.001$.

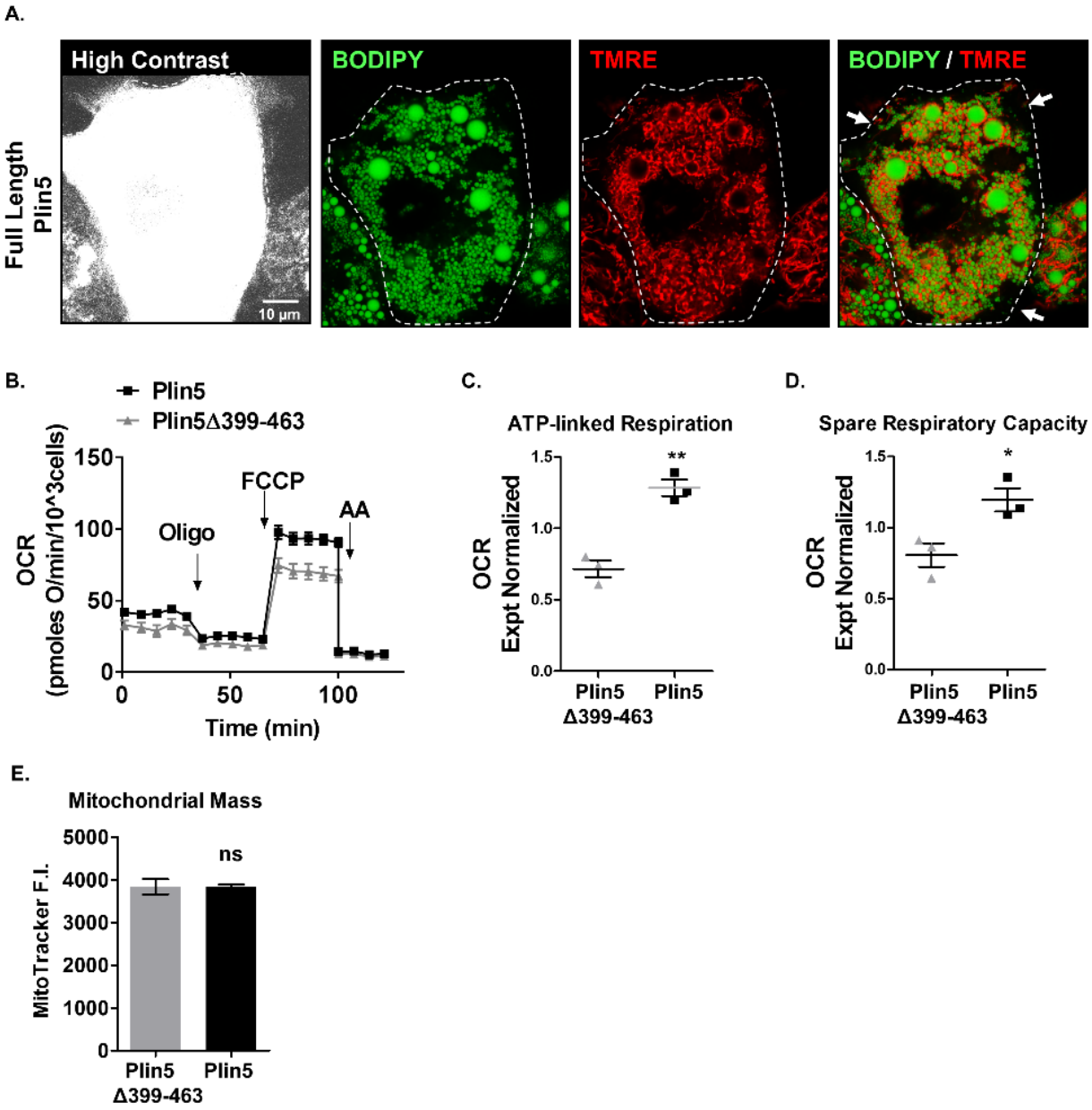

Figure S4, related to Figure 5.

A. Confocal image of brown adipocyte expressing the full length Plin5, which includes mitochondrial recruiting sequence. High contrast image was used to delineate the cell borders (white striped lines). Note that there are several empty cytoplasmic spaces (white arrows).

B-D. Seahorse respirometry in cultured brown adipocytes.

B. Representative trace of oxygen consumption rate (OCR) or cultured brown adipocytes expressing full-length Plin5, which includes the mitochondrial recruiting sequence, and truncated Plin5Δ399-463, which lacks the mitochondrial recruiting sequences as a control. Oligomycin, FCCP, and Antimycin were sequentially injected to assess ATP-linked respiration and spare respiratory capacity. 4-6 technical replicates per group. Data are expressed as means ± SEM.

C. Quantification of ATP-linked respiration in N = 3 independent experiments. Experiment normalized data are presented as means ± SEM. ** $p<0.001$,

D. Quantification of spare respiratory capacity in N = 3 independent experiments. Experiment normalized data are presented as means ± SEM. * $p<0.05$.

E. Controlling for mitochondrial mass. Quantification of mitochondrial mass in cells used for seahorse analysis by MitoTracker staining. ns $p > 0.05$.

Figure 5S

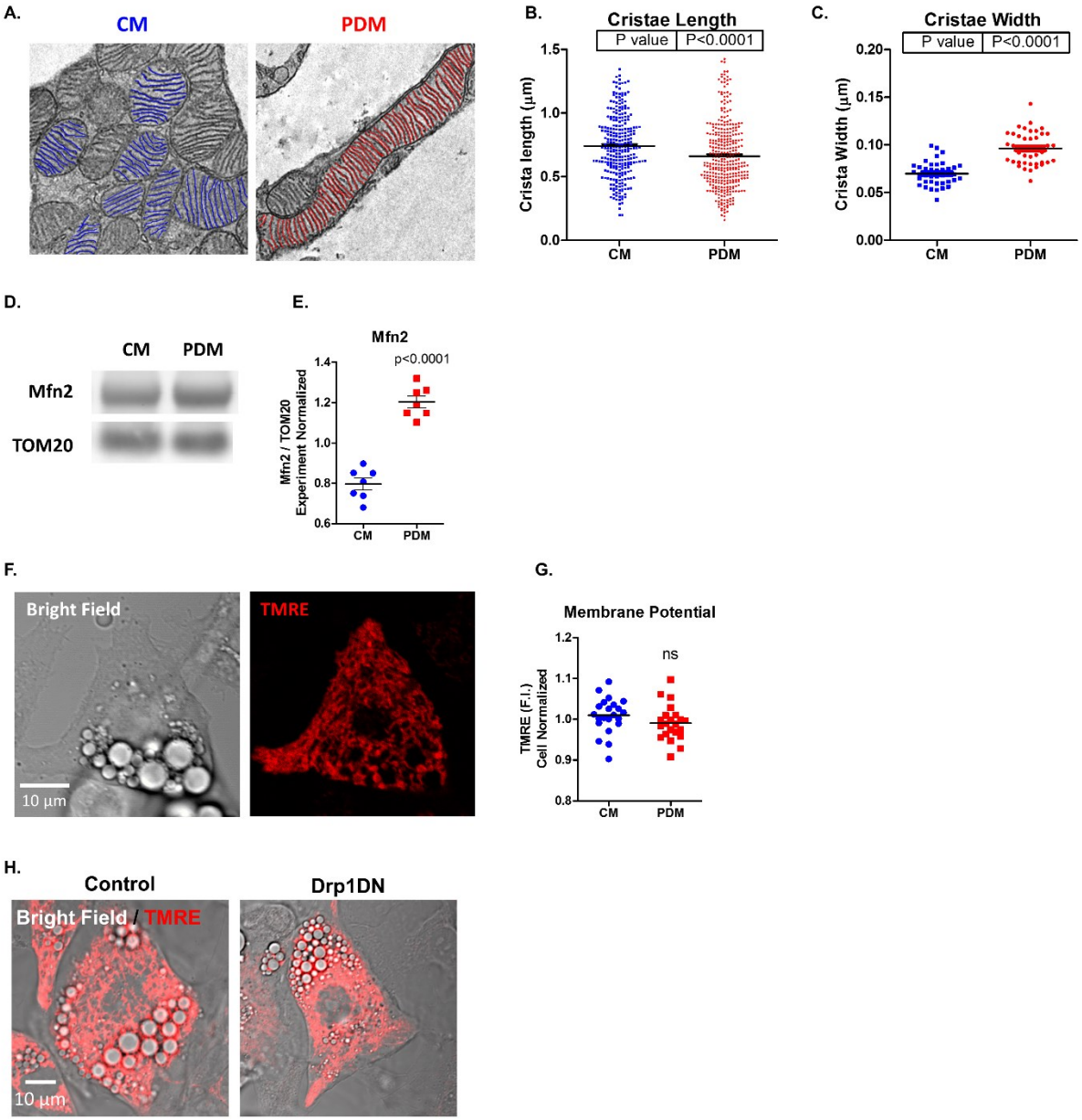

Figure S5, related to Figure 7.

A-C. Cristae morphology analysis of peridroplet (PDM) and cytoplasmic (CM) mitochondria in electron micrographs of BAT harvested from mice adapted to thermoneutral environment (28°C), where PDM are most abundant. Note the uniform stacks of short thick cristae in PDM that are arranged in perpendicular orientation to the axis of mitochondria-LD interface.

D-E. Western blot analysis of Mitofusin2 (Mfn2) in isolated PDM and CM. N = 7 independent mitochondrial isolations. For each individual experiment, average values of CM and PDM were normalized to the average OCR of all mitochondria (see Quantification and Statistical Analysis for complete equations). Data are presented as means ± SEM.

F-I. Imaging of the membrane potential-sensitive dye TMRE. N = 22 cells images in 4 independent experiments. CM and PDM fluorescent intensities (F.I.) were normalized to average cell F.I. for each individual cell. Data are presented as means ± SEM. ns $p>0.05$.

H. Confocal images of living primary brown adipocytes transduced with Drp1-dominant negative (Drp1DN) and transduction control. LDs were identified by bright field images and the mitochondrial network was marked with TMRE.